Force Spectroscopy of the Double-Tethered ConcanavalinA Mannose Bond

Towards Single-Molecule Presymptomatic Detection of Disease

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Abstract:

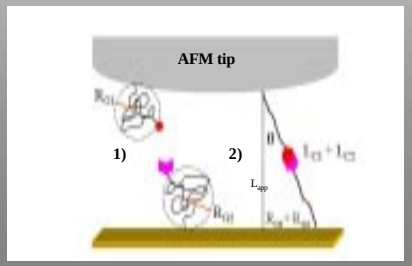
A large number of biological recognition mechanisms involve ligands and receptors that are tethered rather than rigidly bound to cell surfaces. We have used Atomic Force Spectroscopy to measure the force-distance interaction between ConA and mannose, both attached to solid supports using polymer tethers. We find that tethering both molecules makes it possible to distinguish bond breaking associated with specific interactions from that associated with non-specific binding by observing the rupture distance along the force-distance curve. Additionally, by measuring both the distribution of rupture distances (with force spectroscopy) and the polymer tether length distributions (with mass spectrometry) and comparing these results to Monte Carlo simulations, we find that the width of the rupture distance distribution correlates well with the width of the distribution of polymer tether lengths convolved with the angle over which the tethers can sample.

Background and Significance:

Molecular recognition in biological systems, such as between receptors and ligands or proteins and antibodies, is the first step in a wide variety of biological processes including bacterial or viral infection. Because infections begin with very low levels of bacterial or viral molecules (i.e. one molecule) binding to cell membranes, recognition spectroscopy has the ability to identify an infection much earlier than all current methods of detection, and long before disease symptoms appear. We have replicated a biologically relevant tethered system by attaching our protein of interest to an AFM cantilever with a flexible polymer as well as tethering the ligand to a solid support. We show that single molecule force spectroscopy combined with Monte Carlo simulations can be used to determine molecular configurations, as well as bond rupture forces of tethered protein-ligand interactions. The results of this work serve as a baseline for moving the technique onto cell surfaces, as well as helping to develop an understanding of how molecules tethered to solid supports behave (e.g. for use in biosensor applications.)

Figure 1:

A schematic of the molecular configuration of the binding system. During an experiment, a functionalized afm tip (shown in grey) is moved into contact with a surface and retracted. In our experimental system both the protein of interest and the ligand to the protein are attached on the end of long flexible polymers. **1)** shows the system prior to retraction, **2)** shows the system during retraction. θ is the angle that the extended polymers make to the vertical. L_{C1} and L_{C2} are the fully extended contour lengths of the polymers on the afm tip and the polymers on the substrate respectively. L_{app} is the projection of the extended linked polymers along the vertical axis. Note that because we only measure the change in position along the vertical axis when acquiring data in force mode, L_{app} is the apparent length at which we measure interactions. L_{app} is dependent on the angle, θ, as well as L_{C1} and L_{C2}.



Results and Discussion:

Figure 2:

Cartoon series showing the multiple interactions that take place during a single tip retraction (many hundreds of force curves are analyzed to generate the histograms shown in figures 4-6.) The colored boxes surrounding the step numbers correlate with the colored arrows (denoting non-instantaneous processes) and colored asterixes (denoting processes whose time scales are too fast to be measured with this technique.)

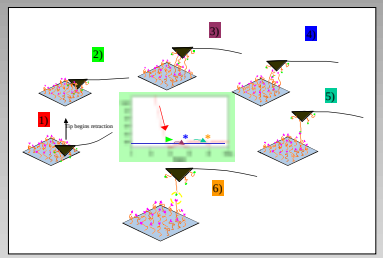


Figure 3:

Experimental AFM retraction curves showing a variety of interactions at a variety of interaction distances. Note that the zero point along the x-axis denotes the point of contact between the tip and the substrate. The data has been rescaled in the y-axis in order to fit all curves on one graph.

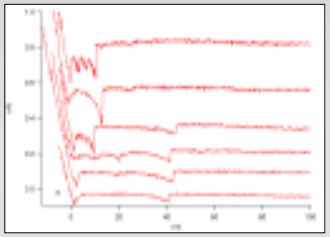


Figure 4:

Experimental AFM retraction curves and proposed schematics showing interactions at three characteristic distances, **A,** non-specific tip-substrate interactions (< 10 nm), **B**, non-specific tip-ligand interactions (between 10-30 nm), and **C**, specific protein-ligand interactions (between 30-40 nm). Note that the zero point along the x-axis denotes the point of contact between the tip and the substrate. Also, force curves **A** and **C** have been rescaled in the y-axis in order to fit all three curves on one graph.

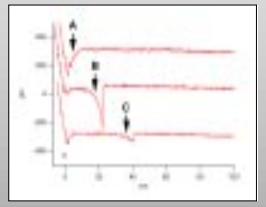


Figure 5:

Histograms of the frequency of interactions (Fig.A) and magnitude of the rupture forces (Fig.B) at increasing distances from the point of contact between the AFM tip and the surface. The distances at which a high number of interactions are seen correlate directly with the distances indicated in Fig. 4.

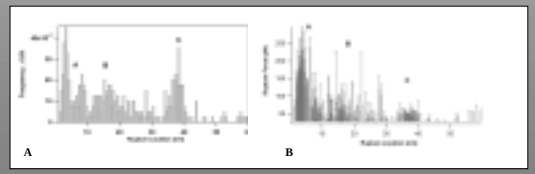


Figure 6:

Histograms of the frequency of interactions (Fig.A and B) and magnitude of the rupture forces (Fig.C and D) at increasing distances from the point of contact between the AFM tip and the surface. Fig. A and C show the specific interactions between the ConA and mannose at the characteristic length scales between ~33 and 43 nm. When the buffer solution in the AFM liquid cell is exchanged with buffer containing free mannose, binding between the ConA on the afm tip and the mannose attached to the substrate is blocked, and the specific interactions seen at the characteristic length scales between 33 and 43 nm can no longer be seen (Fig. B and D)

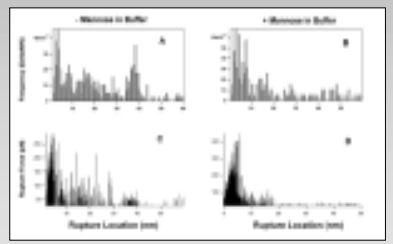


Figure 7:

Rupture force histograms showing the distribution of forces from the three characteristic regions of the force curves. Figure A shows the distribution of forces measured between 33 and 43 nm from the point of contact between the tip and substrate. A Gaussian fit to the histogram reveals the single molecule rupture force for the ConA-mannose interaction of 44 ± 10 pN. Note that this force was measured at a bond loading rate of 100 nm/second.

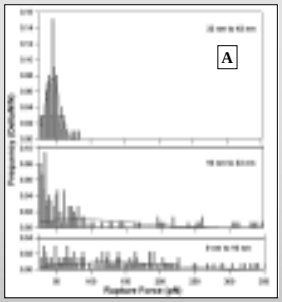


Figure 8:

A) The distribution of bond angles at the instant of bond rupture calculated using a freely-jointed polymer model for each PEG. As the contour length of the PEG (measured in units of the persistence length, i.e. links in the model) increases, the ratio decreases as 1/sqrt(N), where N is the number of links. This trend to small angles suggests that long PEG tethers act to limit unwanted angle dispersion effects.

B) The distribution of bond angles convolved into length distributions as $L_{app} = L_C(\cos\theta)$.

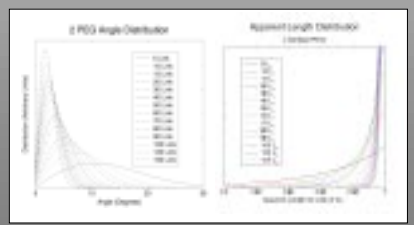
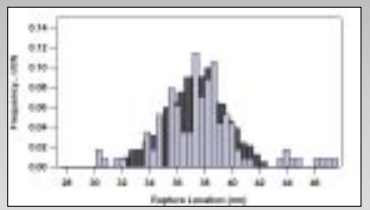


Figure 8:

Experimental (grey bars) and simulated (black bars) histograms showing the width of the length distribution for the specific interactions between tethered ConA and tethered mannose. For the simulation, the system included a single fixed length tether on the afm tip and a range of tether lengths (taken from the mass spectrometry data) on the substrate.



Conclusions

Specific vs nonspecific bonds can be distinguished by location, distribution, and blocking experiments.

Configurational aspects of the system can be determined from the distribution of rupture locations (i.e. one tether vs multiple tethers, and the distribution of ligand length scales.

The result is a length distribution whose width is dominated by the intrinsic width in the PEG length distribution, spread by only about 1 nm due to the angle distribution for the long polymer chains of interest consisting of about 77 monomers each.

Future directions

We are beginning to investigate the use of SHALS (Synthetic High Affinity Ligands) functionalized onto AFM tips for the purposes of detecting diseases in living cells and infectious or toxic agents in solution.The two SHALs that are available to us through Rod Balhorn (BBRP) are designed to be specific for TetC (a simulant for botulinum)and HLADR10, a protein that s heavily expressed in some cancer cells., a Additionally, with Christine Orme- a scientist in CMS- we are investigating protein adsorption to crystal faces, a process that is involved in biomineralization.

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–Biometrology, Inc.
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–Physics, ASU

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• Force curves were analyzed using a collection of modified macros originally written for Igor Pro by Dmitri Venezov.

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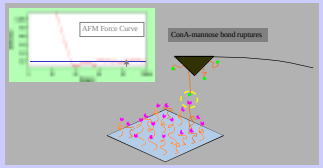
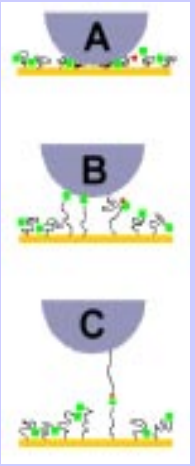
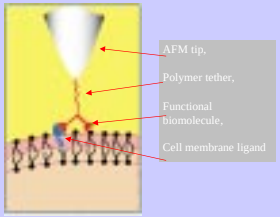
Molecular Recognition Force Spectroscopy involves attaching proteins or other biologically significant molecules to the tip of an AFM cantilever via a polyethylene-glycol tether, allowing the tip to contact and bind with ligands in a cell membrane or bio-mimetic surface, and then retracting the tip from the surface. As the tip is retracted the force required to break the bond formed between the protein on the tip and the ligand on the cell surface is measured. If the ligand that is recognized by the protein on the AFM tip is not present at the surface (e.g. the cell is not infected) then no specific bond is formed. The flexible tether serves an important purpose by moving the protein-ligand bond-breaking event that is monitored by the AFM away from the surface of the tip, as well as increasing the area over which the protein can detect a ligand.







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